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Review

Tetrahydrobiopterin and endothelial nitric oxide synthase activity

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1. Introduction

Nitric oxide (NO) plays a crucial role in the regulation of vascular tone and maintenance of vascular integrity [1–3]. Indeed, nitric oxide reduces vascular tone, inhibits leucocytes adhesion to the endothelium, platelet-vessel wall interaction, as well as vascular smooth muscle cell proliferation and migration. Accordingly, major risk factors for atherosclerotic vascular disease such as hypercholesterolemia, diabetes, hypertension and smoking, have been associated with impaired nitric oxide activity [4–11].

In vivo the activity of the L-arginine-NO pathway is a balance between the synthesis and breakdown of NO. Although, there are several reasons to believe that NO synthesis could be impaired [12–14], reduced NO activity could be caused by enhanced catabolism. Indeed, the in vivo half-life of NO is determined mainly by its reaction with oxyhemoglobin and superoxide anion [15]. Superoxide (O_2^-) may rapidly react with NO to produce peroxynitrite ($OONO^-$; [16]). This reaction is even faster than the one of O_2^- with superoxide dismutase to form hydrogen peroxide (H_2O_2) and molecular oxygen. High concentrations of $ONOO^-$ are very toxic, $ONNO^-$ can form peroxynitrous acid whose cleavage products are among the most reactive and damaging species in biological systems [17]. Taken together, these data indicate that catabolism of NO by its reaction with superoxide could be an important mechanism underlying endothelial dysfunction and oxidative vascular injury described in a number of vascular diseases [18,19]. It can be postulated that harmful concentrations of $ONOO^-$ can be achieved in a dysfunctional endothelium in which O_2^- generation is increased by cyclooxygenase, xanthine oxidase, and NADH oxidoreductase [20–22]. However, recent evidence indicates that decreased availability of tetrahydrobiopterin may be re-

sponsible for a dysfunction of nitric oxide synthase leading to a shift in the balance between the production of protective NO and deleterious oxygen-derived free radicals (Fig. 1). Tetrahydrobiopterin is known to be a cofactor of aromatic amino acid monooxygenases, which are regarded as key enzymes in the biosynthesis of several neurotransmitters, including catecholamines and serotonin [23]. It is indeed well established that inborn errors of tetrahydrobiopterin metabolism lead to cofactor deficiency, hyperphenylalaninemia, and neurological impairment [24]. In contrast, an important role of tetrahydrobiopterin in cardiovascular system has been recognized only recently. The first step of tetrahydrobiopterin biosynthesis involves activation of guanosine triphosphate (GTP)-cyclohydrolase I, which catalyzes the conversion of GTP to dihydroneopterin triphosphate [25]. Intracellular levels of tetrahydrobiopterin can also be increased by treating cells or animals with sepiapterin, which is converted to tetrahydrobiopterin via the so-called 'salvage pathway'. This review will briefly discuss the complex interaction between tetrahydrobiopterin and nitric oxide synthase and its role in the control of vascular tone.

2. Effects of tetrahydrobiopterin on NOS activity

NO is synthesized from L-arginine by nitric oxide synthase (NOS) through a five electron oxidation. Three distinct NOS isoforms have been identified by molecular cloning [26]. Two of them are expressed constitutively in neurones (neuronal) and vascular endothelial cells (endothelial) and are activated by increased intracellular calcium levels. The expression of a third isoform is induced, in a calcium-independent fashion, by various cytokines in macrophages and a number of other nucleated mammalian cells including hepatocytes and vascular smooth muscle

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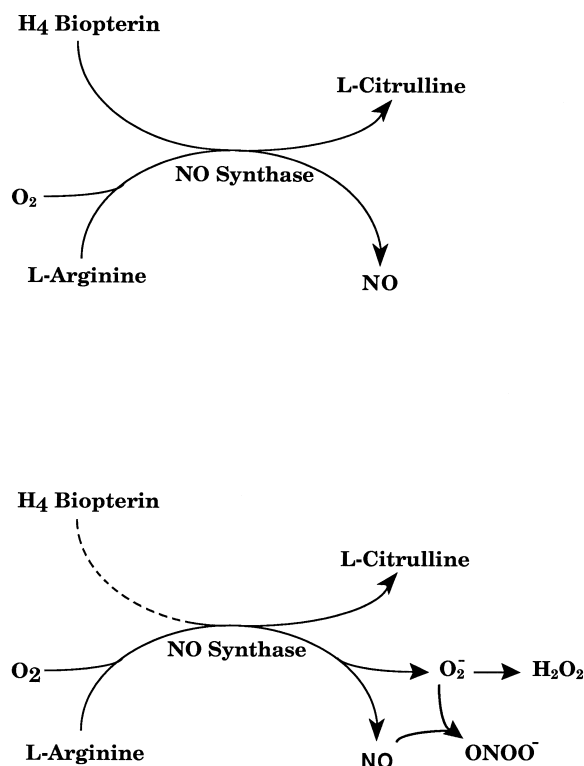


Fig. 1. Schematic representation of nitric oxide synthase (NO synthase) reaction leading to L-citrulline and nitric oxide (NO) from L-arginine and oxygen (O_2) (top). The activation of NO synthase at suboptimal levels (dashed line) of (6*R*)-5,6,7,8-tetrahydrobiopterin ($\text{H}_4 \text{ Biopterin}$) generates superoxide anion (O_2^-) followed by the production of hydrogen peroxide (H_2O_2) and/or peroxynitrite (ONOO^-) from the rapid reaction of O_2^- and NO (bottom) (modified from ref. [38]).

(inducible). Purification and cloning of NOS isoforms have revealed that they are self-sufficient one-component cytochrome P-450s, which contain a prosthetic heme group catalyzing the reductive activation of molecular oxygen requisite for L-arginine oxidation, as well as tightly bound flavins shuttling NADPH-derived electrons to the heme (Fig. 2; [27]). Classical cytochrome P-450 hydroxylating systems would operate perfectly with these cofactors being

properly bound, but NO synthases do not. All three NOS isoforms additionally require tetrahydrobiopterin for catalytic activity. Tetrahydrobiopterin (H_4B) appears to mediate coupling of oxygen reduction to heme-catalyzed L-arginine oxidation to form NO and L-citrulline, but the molecular mechanism of this effect is still unknown. It could involve either an allosteric effect on the NOS protein or redox activity of H_4B , or both [25]. A close link between cellular H_4B availability and NO synthesis was recently demonstrated for a number of different cell types: murine endothelial cells, vascular smooth muscle cells, porcine and human endothelial cells, suggesting that the pathways for H_4B and NO synthesis are tightly coupled [25]. Indeed, in porcine and human vascular endothelial cells, inhibition of H_4B synthesis reduces production of NO in response to the calcium ionophore A23187 or bradykinin [28,29]. These studies provided evidence that in cultured endothelial cells, an optimal concentration of tetrahydrobiopterin is essential for agonist-induced, calcium-dependent production of nitric oxide.

Although, the precise role of this cofactor in regulation of NOS catalytic activity is still not completely understood [25], it has been postulated that H_4B plays an important role in whether the electron flow, within the enzyme, can be directed to L-arginine. Indeed, several biochemical studies demonstrated that activation of purified constitutive NOS in the presence of suboptimal levels of tetrahydrobiopterin results in uncoupling of oxygen reduction and arginine oxidation, thereby generating superoxide anions and subsequently hydrogen peroxide [30–32]. In agreement with these results, we recently demonstrated that in isolated canine coronary arteries depleted of tetrahydrobiopterin endothelial nitric oxide synthase may become a source of oxygen-free radicals [33]. Ever since, growing evidence indicates that, under certain pathological conditions, decreased tetrahydrobiopterin availability may be responsible for dysfunction of endothelial NOS.

Very intriguing are recent findings showing that endothelial cells that were incubated with LDL released superoxide, which could be inhibited by the NOS inhibitor

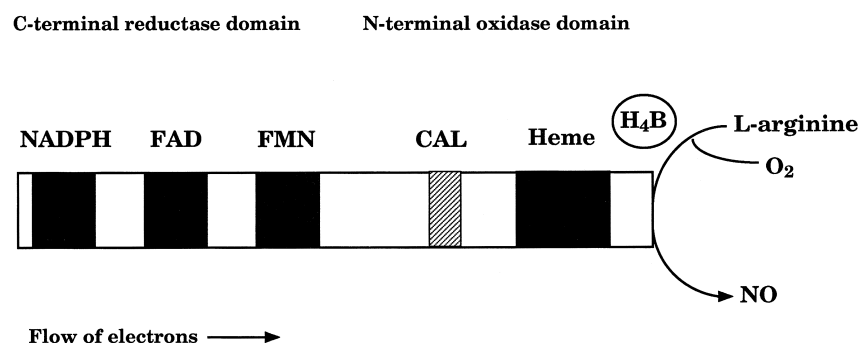


Fig. 2. NO is produced by NOS, which incorporates molecular oxygen into the substrate L-arginine. The NOS itself has binding sites for tetrahydrobiopterin (H_4B), L-arginine and heme. Electrons donated by reduced nicotinamide-adenine dinucleotide phosphate (NADPH) are shuttled through the reduced flavins, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) toward the oxidase domain. The built-in electron transport system is used to oxidize L-arginine to NO and L-citrulline. This reaction is dependent on the presence of H_4B .

L-NAME [34]. These data suggest that NOS itself can be an important source for endothelial superoxide production in hypercholesterolemia. Indeed, enhanced oxidative degradation of NO is a major determinant of impaired NO activity in hypercholesterolemia [35–37]. Deficiency of tetrahydrobiopterin causes both impaired NO activity and increased oxygen radical formation [33,38]. In this regard, we further demonstrated that infusion of tetrahydrobiopterin into the brachial artery of patients with hypercholesterolemia restores endothelial dysfunction by increasing production of NO [39]. Therefore, increased breakdown of nitric oxide could be explained from a decreased availability of tetrahydrobiopterin. Cofactor supplementation may restore NO activity by decreasing oxygen radical formation. Tetrahydrobiopterin can also improve abnormal endothelium-dependent coronary vasomotion in response to acetylcholine in patients with coronary artery disease [40]. Furthermore, administration of tetrahydrobiopterin is capable of restoring endothelium-dependent vasodilation in experimental diabetes [41], smoking [42,43], and reperfu-

sion injury [44]. Such effect of exogenous tetrahydrobiopterin is consistent with the concept of an altered tetrahydrobiopterin-NOS interaction which may lead to the above-mentioned dysfunctional activity of the enzyme.

Interestingly enough, an impaired synthesis of H_4B occurs in adrenal cortex of spontaneously hypertensive rats (SHR; [45]). This metabolic dysfunction was detected in prehypertensive animals suggesting that it may contribute to the development of hypertension and/or its complications. We reported [38] that in isolated aortas from prehypertensive SHR, H_4B supplementation diminished the NOS-dependent generation of superoxide and its dismutase product hydrogen peroxide, while it increased the net production of NO (Fig. 3). Although, the levels of H_4B from SHR were not different when compared to Wistar-Kyoto (WKY) aortas, NOS activity in response to exogenous H_4B was significantly higher in the latter. These results suggest that an increased requirement for H_4B may trigger an uncoupling of the oxidative and reductive domain of the enzyme resulting in dysfunctional

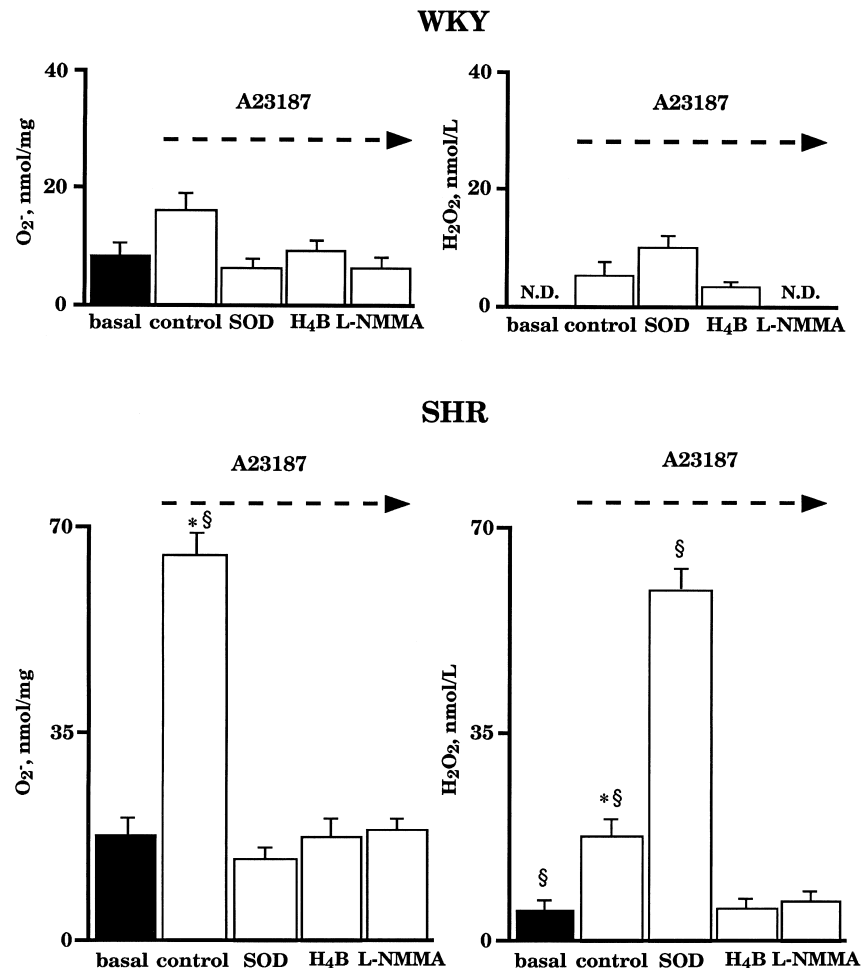


Fig. 3. Bar graphs showing the basal and calcium ionophore A23187-stimulated concentration of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) in aortic tissue from 4-week old Wistar-Kyoto rats (WKY, top) and spontaneously hypertensive rats (SHR, bottom). Note that after tetrahydrobiopterin (H_4B) supplementation and in the presence of NOS inhibitor N^G -monomethyl-L-arginine (L-NMMA) the A23187-induced concentrations of O_2^- and its dismutase product H_2O_2 were significantly reduced only in SHR (reprinted from ref. [38]).

NOS activity. Whether oxygen free radicals formed via NOS plays a role in the development of hypertension remains to be determined.

3. Conclusions

All together these observations strongly support the concept of a dysfunctional NOS as a new source of reactive oxygen metabolites. This NOS-catalyzed formation of O_2^- and its subsequent transformation into HONOO cleavage products, or its dismutation into H_2O_2 and Fenton reaction product OH, may play a pivotal role in the endothelial dysfunction and oxidative vascular injury described in a number of vascular diseases. Therefore, reduced availability of H_4B may represent an important mechanism underlying conditions associated with impaired NO activity and accelerated atherosclerosis. Although the background for such a deficiency is not clear, these findings warrant further exploration for a better understanding of signal transduction pathways sustaining the formation of H_4B in the endothelium. The present knowledge not only underscore the relevance of H_4B as crucial cofactor for NO synthesis but also may initiate research into new therapeutic approaches to reduce cardiovascular risk.

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